Resistance of E. coli to ampicillin using lux and pUC18 plasmids

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Abstract

This experiment was carried out to study the transformation of E. coli artificially using pUC18 and lux plasmids. These plasmids both contain genes which code for resistance to antibiotic in bacteria. The aim of the study was to get an E. coli new strand which is resistance to ampicillin in an environment that is controlled. A sample of the bacteria in question was prepared using a process called competency which enables the cells to easily assimilate the plasmids that are resistant to the antibiotic. To reach competency the bacteria is washed with $CaCl_2$ in an ice bath.

The used solution is placed in a water bath that is warm for heat shocking with an aim of ensuring the cell membrane contracts and expands to allow easy uptake of the plasmids. The bacteria that resulted out of this were then spread on agar plates that had nutrients allowing incubation and growth. After 24 hours of incubation at 37℃ the results were analyzed and images recorded. Signs of growth were evident on agar plates with plasmids and no colonies on plates with no plasmids. This implies that the bacteria picked up the plasmids DNA and were able to resists ampicillin presence.

Introduction

Most of the numerous strains of *E.coli* bacteria are harmless and occur naturally in the intestines of animals however there are those that produce toxins that are powerful and thus able to cause severe illnesses which may include kidney failures and diarrhea (Taketo, 2008). The creation of antibiotics is therefore aimed at reducing the risks of these harmful bacteria like E. coli that cause diseases. Ampicillin as an antibiotic is good at preventing the growth of *E.coli* when exposed to it. For *E.coli* to grow and survive as well as reproduce in the presence of

ampicillin the bacteria is supposed to get DNA molecules which are in existence separately from the chromosomes of the nucleoid region of the bacteria these are called plasmids.

The plasmids are capable of carrying genes which once expressed they assist the bacteria to survive and still multiply when exposed to antibiotics like ampicillin. Ampicillin is an antibiotic that confers resistance to both positive and negative bacteria present in nature. However, the effectiveness of this antibiotic to the management of *E. coli* infections has called for the need to find out the root cause (Spengler et al., 2006). It's evident that some stains of E. coli have the ability to pick up plasmids which possess genes for resistance to ampicillin. These plasmids include p UC18 and lux plasmid. A study of these plasmids and their effect on the ampicillin drug is necessary so as to find different ways of counteraction and also to carry out modifications on ampicillin so that it becomes effective against E. coli.

The blue print of life has been said to be DNA. Transcription of DNA to RNA allows for the blue print present in DNA to be easily read by machinery which is translational in nature. This converts it into protein that is understood by the cell. The plasmids in such experiments serve as the DNAs (Bennett et al., 2004). In as much as plasmids lack necessity in life, they in most cases give the bacteria some advantages in situations like surviving in the presence of antibiotics. Plasmids like lux and Puc18 have been reported to promote growth of bacteria in agar media that has ampicillin in it. This implies that they assist the bacteria to grow resistance to the antibiotic. An ampicillin resistance gene exists in Puc18 which is known to facilitate growth of *E.coli* while lux forms part of the bioluminescent bacterium *Vibrio Fischeri*.

Any bioluminescent plasmid is known to posses' enzyme luciferase that makes it emit light. For successful transformation to take place a host has to exist so as to be able to deliver the DNA in the cell and also means to identify these cells which have undergone transformation

have to be used. Puc18 and lux act as vectors during transformation exercise as *E.coli* acts as a host. Once transformation is successful the bacteria can grow in the presence of ampicillin. Lawn and colonial growth need to be present and visible on the agar plates in order to determine the success of this transformation (Taketo, 2008). Incase lawn and colonial growth do not appear then it's an indication that ampicillin is present and acting on the bacteria.

Such an outcome allows scientists to determine bacteria that can be used to generate medicine that is used in fighting bacteria diseases. E. coli is present in the digestive system of humans and it produces vitamin K, however it too much exposure to it can lead to urinary tract infections, gastroenteritis and meningitis. By use of transformation in introducing foreign DNA into the *E.coli* scientists are able to understand the pathogenic nature of *E. coli.* The experiment is therefore crucial in assisting scientists to get ways in which foreign DNA can be introduced in a complex eukaryotic cell. Plasmids exist in most bacterial species and they are circular DNA molecules small in size. In many cases they are not of great importance especially for survival of the bacteria. They however have genes which assist the bacteria to survive in specific conditions like exposure to antibiotics (Toleman, Bennett & Walsh, 2006).

Some plasmids are seen having genes which confer resistance to antibiotics and therefore if a bacteria cell has such a plasmid it will live and undergo multiplication in presence of any antibiotic. Most of antibiotic resistant *E.coli* that have been isolated in different parts of the world have been known to possess plasmids of this nature. They carry genetic information for the products of proteins which have the capacity to interfere with antibiotic actions. pUC18 contains 2,5686 nucleotide pairs with a molecular weight of 2x106. The plasmid is small in size and therefore less susceptible to any physical damage.

Being small in size also implies that this plasmid replicates very efficiently in the bacteria producing large numbers of plasmids in each cell. Copies of this plasmid may be as many as 500in one cell of *E.coli (*Levy, 2002). The plasmid has an ampicillin resistant gene that makes *E.coli* to grow in the presence of ampicillin. The resistance gene of ampicillin in this plasmid codes for beta-lactamase enzyme that is able to inactivate ampicillin and even penicillin. Transformation is used to introduce plasmids in a bacterial cell. Once placed in a solution of calcium chloride the bacteria are able to take in the DNA molecules of the plasmid. Through this procedure large amounts of DNA plasmid that is specific can be prepared.

Any one transformed cell is capable of giving rise to clones which have the same replica of the parent DNA plasmid molecule. Once bacteria have grown in the presence of antibiotic the DNA for the plasmid is readily isolated from the culture of bacteria. For molecular biologists plasmids are useful tools because they serve as carriers for genes. A procedure used in recombinant DNA technology is made up of the joining of a gene to the DNA plasmid so as to form a hybrid or a recombinant molecule (Toleman, Bennett & Walsh, 2006). This molecule should be able to replicate in the bacteria. For preparation of recombinant molecules plasmid and gene are cut and spliced together at precise molecules. Once the preparation of the hybrid plasmid molecule is done, it then gets introduced into the *E.coli* by transformation procedure.

While the bacteria cells divide, the plasmid hybrid also replicates to yield many copies of the original gene. Once growth is done, the purification of the hybrid molecules from the bacteria is done and recovery of the original gene takes place. Using this method, scientists have been able to obtain large quantities of close to 1,000 specific genes among them being genes used for insulin and growth hormones in human beings.

Material and procedure

Materials-plasmid lux, control plasmid (p UC18), petri dishes with ampicillin nutrient agar, nutrient broth, inoculating loops, sterile transfer pipettes, sterile tubes, calcium chloride, E. coli, water bath, ice bath, light free room, sterile micropipettes, and bleach solution or any disinfectant (Taketo, 2008).

Procedure

During this study plasmid pUC18 which is the control plasmid and lux plasmids get introduced into the *E.coli* using transformation process using the steps below;

-bacterial cells treated with calcium chloride solution so as to ensure uptake of the plasmid DNA is enhanced. Once treated with calcium chloride they are said to be competent.

The competent cells are then incubated with plasmid DNA.

Cells that will have taken up the DNA of the plasmid grown on ampicillin containing medium

The culture is then examined in the dark

A vial of calcium chloride solution is placed in the ice bath together with *E.coli* tube. With a sterile pipette 0.5 m L of calcium chloride is transferred to the tube which has the bacteria.

The contents of the tube are then transferred to a vial using the same pipette which has most of the calcium chloride solution (Bennett et al., 2004). Using the tip of the index finger the vial is tapped to allow the solution to mix.

The cells are incubated for 10 minutes on ice. The cells once are able to take up the DNA from the medium are said to be competent. The cells can be stored in calcium chloride solution for 24 hours if need be.

DNA uptake by competent cells

Small tubes are labeled i.e. ‘C DNA’ for control and ‘L DNA’ for the lux plasmid. The tubes are then placed in an ice bath. 10 u L of the control plasmid is added to the ‘C DNA’ tube using a sterile micropipette and 10 u L of the lux plasmid is added to the ‘L DNA’ tube.

To ensure the cells are in suspension, the tube of competent cells is gently tapped with the index finger tip. With a sterile pipette 6 drops of competent cells are added to each tube. Using the index finger the tubes are tapped for mixing of the contents and stored on ice for approximately 15 minutes (Spengler et al., 2006). The competent cells suspended in the calcium chloride solution then starts taking up the DNA of the plasmids. Two additional tubes are used in which 6 drops of the competent cells are added and labeled ‘NP’ for no plasmids.

The tubes are transferred to a water bath that has been preheat to 37C and allowed to remain there for 5 minutes.

A sterile pipette is used to add about 0.7 m L of nutrient broth to the tubes and incubation done at 37C for 45 minutes. This incubation period is to allow time for bacteria to recover from the treatment of calcium chloride and start expressing its resistance to ampicillin using the gene present on the plasmid.

Selection of cells which have taken up plasmid by growing on a medium containing ampicillin

Four ampicillin nutrient agar plates are obtained and one plate is labeled ‘C DNA, another ‘L DNA’ and the other ‘NP’ (Fernández-López et al., 2006).

With a sterile pipette 025 m L of bacteria suspension mixture is gotten from ‘C DNA’ tube with the removal of the lid from this tube and dispensation of bacteria on the agar takes place. An inoculating tube is used for the spreading of bacteria in an even form on the surface of the agar.

The same is repeated by transferring 0.25 m L of bacterial suspension from 'L DNA' plate and using an inoculating tube its spread on the agar block.

Those cells from the tubes with no plasmids are placed on two plates of NP as described in the above steps for L and C DNAs. The lids are replaced on the plates and left at room temperature until absorption of the liquid is completed. This may take 10-15 minutes.

The plates should be inverted and incubated in the light free room at room temperature.

Once the experiment is completed disinfection of the equipment is done using 10% bleach where the pipettes, loops and tubes are immersed in a sink containing the bleach or flooded with the solution. The disinfection is done for 15 minutes and excess bleach drained off and the plastics materials are sealed and disposed of (Avison & Bennett, 2005). This precaution is taken to ensure the E. coli does not get to the environment and contaminate the food or even water which eventually may get into human body and cause infections. The un-disposable ones are autoclaved for further disinfection before being stored for future use.

Discussion

From the experiments upon successful transformation the NP plate which did not have plasmids had a negative growth while the CDNA and LDNA plates showed positive growth. This is an indication that the plasmids are capable of interfering with the activity of ampicillin on bacteria. The fact that they were not introduced on the NP plate it indicates that ampicillin was able to inhibit growth of bacteria on the plates hence no growth was seen. For the C and L plates the presence of lux and p UC18 plasmids hindered activity of ampicillin and thus facilitating growth of bacteria.

phdkingdom.com sample

Colonies appeared 2-3 days when incubated in the dark room and viewing of the plates done at that time this is because delays may lead to failure in bioluminescence because it decreases with time once the colonies have formed. The L DNA plates had bioluminescence colonies because this plasmid has the enzyme responsible for illumination under light. The other two plates did not bioluminescent colonies because they lack this enzyme.

For colony formation close to 200 colonies was evident on the L and C plates an indication that the plasmids are capable of hindering the effectiveness of ampicillin. The luciferase enzyme was responsible for the illumination. This enzyme lacks in plasmid p UC18. In this experiment also it was noted that plasmid size affects efficiency of transformation, and since lux plasmid had larger size than p UC18 then lux showed fewer colonies compared to its counterpart. This is because it is often difficult for the cells to absorb plasmid DNAs with larger sizes. The large size of the plasmid also makes it susceptible to physical damage and lessens the replication efficiency.

Conclusion

From this study it's evident that plasmids p UC18 and lux are capable of increasing the resistance of *E.coli* to ampicillin as seen from the laboratory experiment. Colony growth was evident on the pates that lacked infusion of the plasmid DNA but was abundant on the two plates which had the plasmids. This indicates that ampicillin was able to inhibit growth of bacteria E. coli on the plates that lacked the plasmids because it was supplied with the genes for resistance to ampicillin effects. This technique is good for other studies related to bacteria and drug resistance; because it can be used to identify which microbe shave the plasmids that are hindering effectiveness of a certain drug and how these plasmids can be inactivated for the drug to be effective. The experiment was a success because the process of transformation.

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